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Abstract: The aims of this study were (1) to develop a set-up for co-registering functional near-infrared imaging (fNIRI) and electroencephalography in 15 healthy term neonates (mean gestational age 39.9 weeks, mean postnatal age 2.7 days) which underwent visual flash stimulation during sleep; (2) to optimize fNIRI sensitivity in regard to detectability of hemodynamic responses; and (3) to analyze whether oxyhemoglobin concentration [O2Hb] rises or falls after stimulus onset. fNIRI sensitivity seems to depend mainly on luminance of the visual stimulation. The sensitivity of fNIRI is 63.6%. When hemodynamic response is detected, a rise of [O2Hb] after stimulus onset was shown in 75% and a decrease of [O2Hb] in 25%.

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How to Conduct Studies with Neonates combining Near-infrared Imaging and Electroencephalography

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Abstract One aim was to develop a set-up for co-registering functional near-infrared imaging (fNIRI) and electroencephalography in 15 healthy term neonates (mean gestational age 39.9 weeks, mean postnatal mean age 2.7 days) which underwent visual flash stimulation during sleep. Further aims were (ii) to optimize fNIRI sensitivity in regard to detectability of hemodynamic responses and (iii) to analyse whether oxy-hemoglobin concentration ($[O_2Hb]$) rises or falls after stimulus onset. fNIRI sensitivity seems to depend mainly on luminance of the visual stimulation. The sensitivity of fNIRI is 63.6%. When hemodynamic response is detected a rise of $[O_2Hb]$ after stimulus onset was shown in 75% and a decrease of $[O_2Hb]$ in 25%.

1 Introduction

In newborn infants, hemodynamic responses (HR) to visual stimulation can be detected by fNIRI. However, available literature is not conclusive with regard to fNIRI sensitivity, or whether $[O_2Hb]$ as part of the HR raises or declines after visual stimulus onset [1]. To be able to determine fNIRI sensitivity, electroencephalography (EEG) is utilized as a reference in this experiment. The visual evoked potential (VEP) provides not only an indication of the existence of a response to a visual stimulation; it is also beneficial when neurovascular coupling is examined and it can be used as reference in further analyses of the fast neuronal signal. The aims of this study were (i) to develop, test and optimize a fNIRI-EEG-set-up, (ii) to achieve high fNIRI sensitivity and (iii) to characterize $[O_2Hb]$'s changes ($\Delta[O_2Hb]$) during stimulation.

2 Methods

After assembling and testing the equipment first measurements were conducted. Responses to the visual stimulation in fNIRI and EEG occurred rarely. Therefore the protocol of the experiment had to be revaluated. Throughout more than 30 measurements, different parameters of the protocol were varied, for example positioning and attachment of sensors, the effect of electrode-skin impedance, bedding of the subject, frequency of stimulation and intensity of stimulation. In each individual measurement only one parameter was varied to allow grading the impact on potential responses. No parameter, except intensity of the visual stimulation device, led to a measureable increase in sensitivity. All further descriptions reflect the latest, most successful protocol in regard to sensitivity.

Subjects and protocol: 15 healthy, term neonates (mean gestational age 39.9 weeks (standard deviation (SD) 0.92 weeks), mean postnatal age 2.7 days (SD 0.94 days)) were stimulated visually during sleep. The HR of the primary visual cortex is registered by an fNIRI-sensor whereas the VEP is recorded by EEG.

The protocol of stimulation has a maximum duration of 20 minutes containing periods of stimulation and rest periods. While the duration of rest periods are different (12 s to 32 s) the stimulation periods are constantly 20 s long. Flash frequency is 0.5 Hz and duration is 10 ms. The flash device is held 15 cm in front of the subject's eyes. After 10 minutes of recording, a preliminary data analysis was performed in order to decide whether to position the fNIRI-sensor differently or to proceed with the recording. If the fNIRI-sensor needed to be readjusted, then only data which were acquired after sensor adjustment were included in the final analysis.

Recordings were performed when room light was turned off and without day light. Throughout preparing and recording a conversation takes place between the conductors and the mother to establish a relaxed ambiance which supported continuous sleep of the infant.

Preparation of subject: Minimal handling of the newborn infant was done to avoid wakening the infant. Measurements were scheduled to take place shortly after feeding, the newborn infants were held comfortably by their mother sitting in an armchair. Cushions were provided to support the mother holding her infant.

After explaining the experiment to the mother, the first step was to clean the skin of the electrode placement with pre-warmed NaCl solution. Then abrasive electrode gel was applied gently with cotton swaps. Finally, the self-adhesive foil-electrode was applied on top of the gel. This procedure was repeated for all electrodes. The positions of the electrodes refer to the 10/20 system [2]. C_Z, F_Z, O_Z and F_3 were used. C_Z acted as reference electrode and F_Z as ground electrode. The O_Z electrode was cut to fit beneath the fNIRI-sensor without obscuring any light-sources or detectors. Relatively high electrode-skin impedances, in the range of up to 40 kΩ, were accepted.

The NIRI-sensor was attached in its center at O_Z. It covered an area of 1.8 cm below and above O_Z, and 1.2 cm in directions O_1 and O_2. The fNIRI-sensor was attached to the head with elastic bandages. Several shorter straps of these

bandages were used to ensure the flexible fNIRI-sensor adapts to the form of the infant's head and has good contact to it. When all bandages were placed they covered the head in a cap-like order. This improved the electrode attachment to the skin. If after the experiment electrodes stuck firmly to the hair, almond oil was used to moisten the electrodes and hence facilitate detachment.

Instrumentation and material: The continuous-wave NIRI-device "Multi-Channel-Photometer II" (MCP-II), an in-house development, was employed. Its configuration used for the measurements described here, features the registration of 11 different light-paths at 3 wavelengths with a sampling frequency of 100 Hz by time multiplexing. A more detailed description of the MCP-II has been published previously in [3]. The light-sensor connected to the MCP-II is flexible, biocompatible and easy to disinfect. It covers an area of 3.75 cm × 2.5 cm. The shortest inter-optode distance is 1.25 cm, and the longest one is 3.75 cm. The sensor consists of 4 light sources, each composed of 3 LEDs (750 nm, 800 nm, 875 nm) and 4 silicon based detector diodes. The arrangement of the sources and detectors is shown in figure 1.

The device controlling the visual stimulation is designed as an extension to the MCP-II. It is configurable through software and provides several outputs which can be switched with a maximum frequency of 100 Hz. Here, only the LED-driver output (IC UDN2981A, 15 V_{cc}) was used to switch red LED arrays. The array of the high-luminance device contains 8 high intensity LEDs (dominate wavelength $\lambda = 660$ nm, 600 cd/m^2 at stimulation device). Duration of a flash for visual stimulation is set to 10 ms. The stimulation device is connected to the MCP-II and the EEG device. Each time a flash occurs an event marker is sent to both devices. To derive the partial EEG, the commercially available NicoletOne from VIASYS Healthcare Inc® is utilized. Its Tornado V44 amplifier records data with 2 kHz sampling frequency. The cutoff frequency of the high-pass filter is 0.01 Hz. Connected to the amplifier are Ambu® Blue Sensor NF electrodes. Those are single patient use, Ag/AgCl, solid gel, self-adhesive, ECG foil electrodes. Major advantages are their thinness and the possibility to trim them to a size which enables location beneath the light sensor.

Data analysis: All algorithms used for analyses were implemented in Matlab®. The NIRI device MCP-II provides raw values reflecting the change of light intensity per wavelength and light-path over time. An algorithm converts the raw values into $\Delta[O_2Hb]$ and changes in deoxyhemoglobin concentration (Δ[HHb]) based on the modified Beer-Lambert law [4] using extinction coefficients as given in [5] and the following DPFs extrapolated from data given in [6]: 4.714 at 750 nm, 4.249 at 800 nm, 3.5515 at 875 nm. The unfiltered $\Delta[O_2Hb]$ and Δ[HHb] traces undergo artefact reduction by the MARA approach [7]. Afterwards the artefact-reduced data are high- and low-pass filtered (high-pass: substraction of moving average filter, span 40 s; low-pass: least squares smoothing filter, 1 s framesize, 1st order). To determine whether a HR is present in the data of a subject, its 11 $\Delta[O_2Hb]$ channels are examined independently with a non-parametric test (Wilcoxon) for significant changes ($p < 0.05$) during stimulus onset. Therefore the data of the time interval from -6 seconds to stimulus onset and the interval from 8 seconds to 14 seconds after the onset are paired for all stimulation periods and used for the statistical test. Raw data of the EEG device

are processed for each channel independently. Data are low-pass filtered (100). Due to acceptance of high electrode-skin impedances and the non-shielded electromagnetic environment of measurement, 50 Hz pick-up can be considerable; notch filtering the data is unavoidable. Therefore, and to reduce electrical coupling of the fNIRI-sensor, data are notch-filtered at 50 Hz and 100 Hz. Amplitudes exceeding a threshold of 200 µV are excluded from further processing. In the final step, time triggered block-averages (TTBA) of stimulation events and TTBA of sham events during rest periods are displayed with their standard error mean. Since the existence of VEPs is simple to find, a computerized analysis of the TTBAs has not yet been implemented. Two persons screened the TTBA traces of channel O_Z independently for transients occurring before 500 ms after stimulus onset and exceeding in amplitude all other data within the given time window. Furthermore the amplitude of the transient must exceed the amplitudes of the TTBA resulting from the sham stimulation events in the same time window. When these prerequisites were met, and a trace is defined by both persons as a VEP, the measurement is regarded as containing a response in EEG data.

3 Results

Examples of responses in EEG and fNIRI are shown in figure 2 and figure 3, respectively. In 73.3% of the subjects, VEPs could be detected. In contrast, significant HRs occurred in 46.7%. No HRs were detected without a corresponding VEP. When EEG data are taken as reference for whether stimulation was successful, the sensitivity of fNIRI increases to 63.6%. 75% of the HRs are based on a rise of $\Delta[O_2Hb]$ after stimulation onset, while 25% are on a decrease.

4 Discussion

As shown in this fNIRI study, the gain by the simultaneous EEG is worthwhile the effort. VEPs could be seen only in 73.3 % of subjects; hence it must be considered that not each attempt of stimulation succeeds. And this raises the question of EEG sensitivity for VEP detection. The EEG sensitivity could be improved by better electrode-skin impedances. However, these high impedances were accepted in order not to wake up the infant by preparing more thoroughly the infant's skin. Consequence of high impedances was increased pick-up of electro-magnetically interference, especially in non-shielded environments like the rooms the measurements were performed. Major contribution to the noise was the mains with their characteristic 50 Hz noise. Although the mains interference was attenuated by employing a notch filter during analysis, it could not be excluded completely that a VEP in the noise remained undetected and lead to a false negative result of the analysis. By analysing the EEG data by TTBA of the

amplitudes during rest periods, the EEG analysis was more stringent than if only latency and amplitude of stimulation periods were determined. This, and most likely less luminance of the stimulation device, could contribute to our EEG sensitivity being smaller than 100% as reported by others [8].

The relatively low sensitivity of fNIRI, 46.7%, is based on all infants, but it increases to 63.6% when only the infants are considered which responded to the stimulation by VEP. The latter sensitivity seems reasonable, yet there are much higher numbers are reported literature, as for example 100 % by Hoshi et al. [9]. In contrast to their study, our procedure of stimulation differed widely from theirs [9] by stimulation frequency, the length and distribution of rest periods.

Another interesting circumstance is that, under the assumption of 100% sensitivity and selectivity of EEG analysis, there was no false positive occurrence in fNIRI data.

5 Conclusions

An fNIRI-EEG-set-up was developed and optimized by conducting more than 30 measurements with subjects. That followed successful measurement of 15 subjects without changes of the protocol. It was found that fNIRI sensitivity improved with higher luminance (600 cd/m^2) of the stimulation's light. 63.6% sensitivity was achieved. $\Delta[O_2Hb]$ increased in 75 % and decreased in 25% of significant HRs detected by fNIRI.

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References

1. Wolf M, Greisen G (2009) Advances in near-infrared spectroscopy to study the brain of the preterm and term neonate. Clin Perinatol 36:807–834.
2. Jaspers HH (1958) Report of the committee on methods of clinical examination in electroencephalography. Electroen Clin Neuro 10:370–375.
3. Haensse D, Szabo P, Brown D et al (2005) A new multichannel near infrared spectrophotometry system for functional studies of the brain in adults and neonates. Opt Express 13: 4525–4538.
4. Kocsis L, Herman P, Eke A (2006) The modified Beer-Lambert law revisited. Phys Med Biol 51:N91.
5. http://www.medphys.ucl.ac.uk/research/borl/research/NIR_topics/spectra/spectra.htm
6. Cooper CE, Elwell CE, Meek JH et al (1996) The noninvasive measurement of absolute cerebral deoxyhemoglobin concentration and mean optical path length in the neonatal brain by second derivative near infrared spectroscopy. Pediatr Res 39:32–38.

7. Scholkmann F, Spichtig S, Mühlemann T et al (2010) How to detect and reduce movement artifacts in near-infrared imaging using moving standard deviation and spline interpolation. Physiol Meas 31:649–662.
8. Benavente I, Tamargo P, Tajada N et al (2005) Flash visually evoked potentials in the newborn and their maturation during the first six months of life. Doc Opthalmol 110:255–263.
9. Hoshi Y, Kohri S, Matsumoto Y et al (2000) Hemodynamic responses to photic stimulation in neonates. Pediatr Neurol 23:323–237.

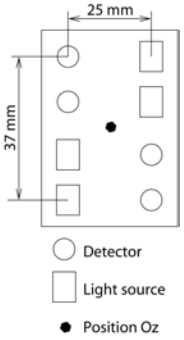
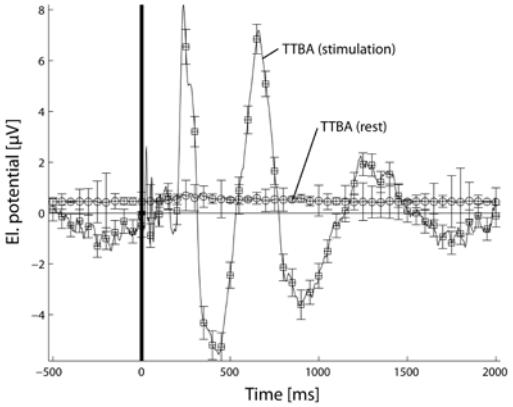


Fig. 1. fNIRI sensor. Interoptode distances and alignment of light sources and detectors.

Fig. 2. TTBA (272 single events) of EEG's channel O_Z. Error bars indicate standard error mean. Stimulus onset is at time 0 s. A clear difference between stimulation and rest periods is noticeable.



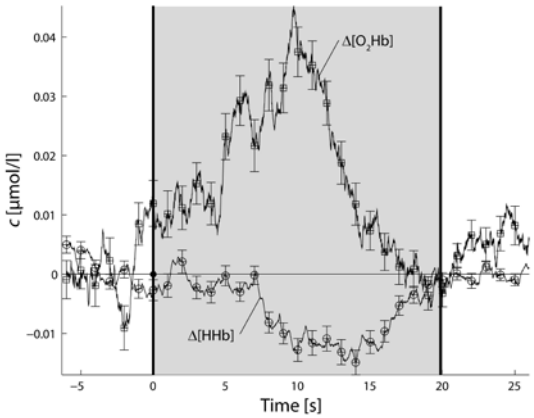


Fig. 3. HR of a single NIRI channel after TTBA over 30 stimulation periods. Error bars indicate standard error mean. Gray shaded area marks interval of stimulation.